

Exhibit B

NOTEBOOK NO. 2233
ISSUED TO R. Santi
ON _____ 19____
DEPARTMENT m3
RETURNED _____ 19____

— SCIENTIFIC NOTEBOOK CO. —
5007 WEST DONNA DRIVE
STEVENSVILLE, MICHIGAN 49127

Obtained ~100 μ l of second prep Tag polymerase from David. (heparin-Sepharose, ~~frac~~ frac 52-53, concentrated)

20 mM Tris-cl pH 8

0.1 M KCl

0.1 mM EDTA

1 mM DTT

50% Glycerol

7400-11,700 u/ml

0.1 Salmon Sperm DNA.

Storage buffer
(note: no gelatin)

Variation in concentration reflects results obtained in two ~~assays~~ assays using different dNTP stocks. Higher value (12 u/ μ l) with assay components used in assay of ~~first~~ first batch Tag polymerase (2167:65).

If variation in u/ μ l depending on DNA substrate is same as before, concentration with M13 primer extension is 21 u/ μ l!

This stuff is ~3x more concentrated than first lot.

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R. Saini

(lot 2)

Assay Tag polymerase for activity in PCR amplification of β -globin ~~using~~ using Molt4 and PC03/04. Vary # cycles (15-30) and amt. enzyme used (1-4 μ l). Also include lot 1 polymerase as control.

15° 20° 25° 30° ~ # cycles

A B C D: 4 μ l lot 1

E F G H: 1 lot 2

I J K L: 2 "

M N O P: 4 "

} 25 μ l per sample
containing 250ng genomic DNA

Molt4 @ 100 μ g/ml, PC03 and PC04 @ 10 μ M, dNTP @ 40mM

50 μ l DNA (5 μ g)50 μ l 10x salts50 μ l 2 μ g/ml gelatin50 μ l PC0350 μ l PC0450 μ l DMSO "75 μ l dNTP125 μ l H₂O500 μ lSplit into four 100 μ l samples.Heat \rightarrow 20', 98°

Cool to room temp and add amounts of enzyme indicated above each

Sub-divide further into four 25 μ l samples and subject to indicated number of cycles. (After overlaying with mineral oil.)

Program: 2 1/2' @ 98°

5' @ 37°

A, E, I, M \rightarrow 14 cyclesB, F, J, N \rightarrow 19 "C, G, K, O \rightarrow 24 "D, H, L, P \rightarrow 29 "

} After last cycle, heat 10-15' @ 60° to finish last ~~exten~~ extension.

Extract mineral oil with CHCl₃ and store at -20°

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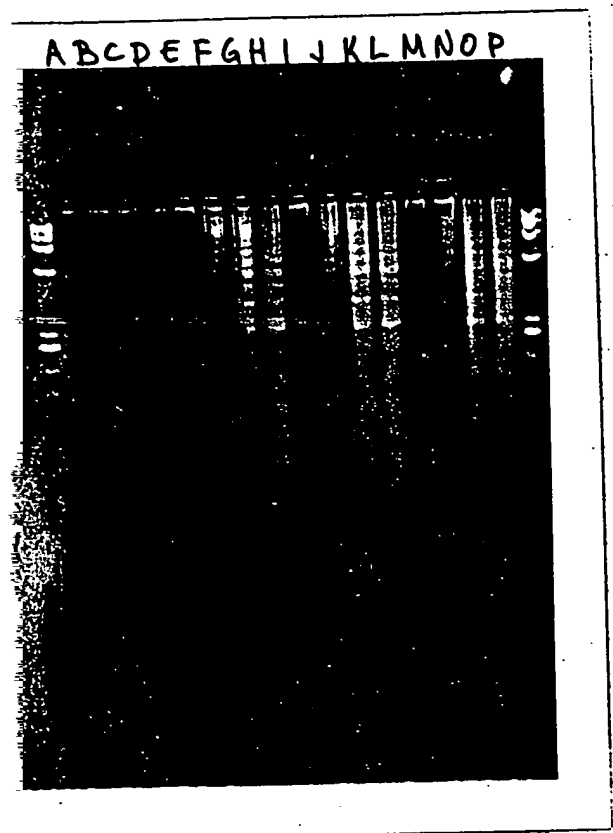
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X

ok samples of Tag polymerase assay on gel and
t if interesting.

As described page 111. Load 5 μ l each sample onto
NuSieve / 0.5% agarose / 1x TBE \rightarrow ON, 15V (~15hr)



15 cycles
20 " 25 " 30 "

A	B	C	D	4 μ l	lot 1
E	F	G	H	1	lot 2
I	J	K	L	2	"
M	N	O	P	4	"

Don't see anything with lot 1 polymerase.
Lots of bands with the new stuff.
Amount of between-band streaking
seems to increase with amt. of
enzyme added. No significant
increase in overall fluorescence
25 vs. 30 cycles even with 4 μ l
lot 2 polymerase. May need to
dilute out (<1 μ l) to reduce
complexity and increase specificity.

This is worth blotting.

Denaturation: ~200ml 0.5N NaOH, 1.5M NaCl \rightarrow 45', RT

Without neutralizing, transfer to Genatran with 20x SSPE
 \rightarrow ON (~16hr)

Wash filter in 20x SSPE and bake \rightarrow 60' @ 80° in vacuum oven.
Label filter 2233:112

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Prepare dot blot containing PCR samples from Tag polymerase assay described page 111.

Apply 5 μ l each sample to filter:

5 μ l	PCR sample
195 μ l	0.4N NaOH, 25mMEDTA
200 μ l	

Rinse each well with 0.4ml 20xSSPE, then entire filter in 20x.

Bake 1 hr @ 80°, in vacuum oven.

Label filter 2233:113

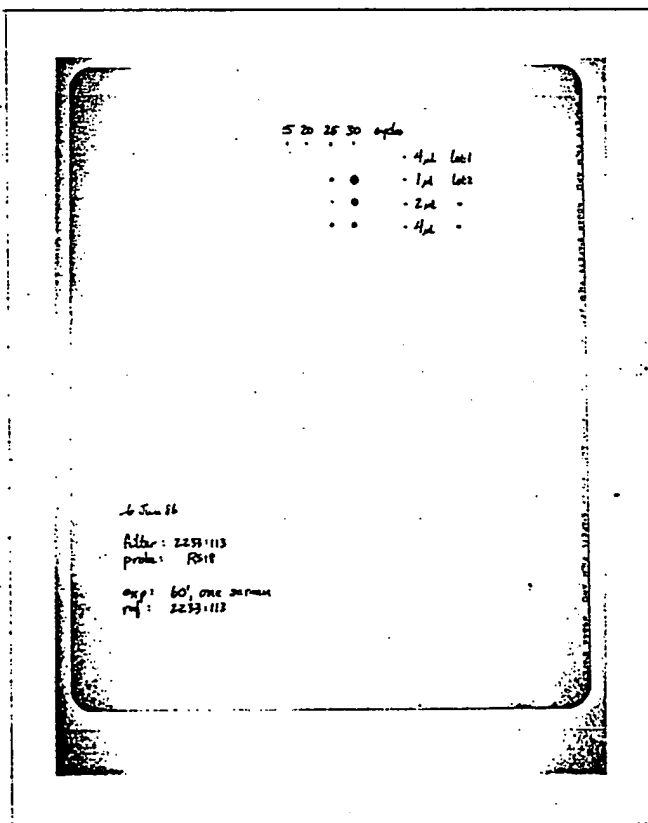
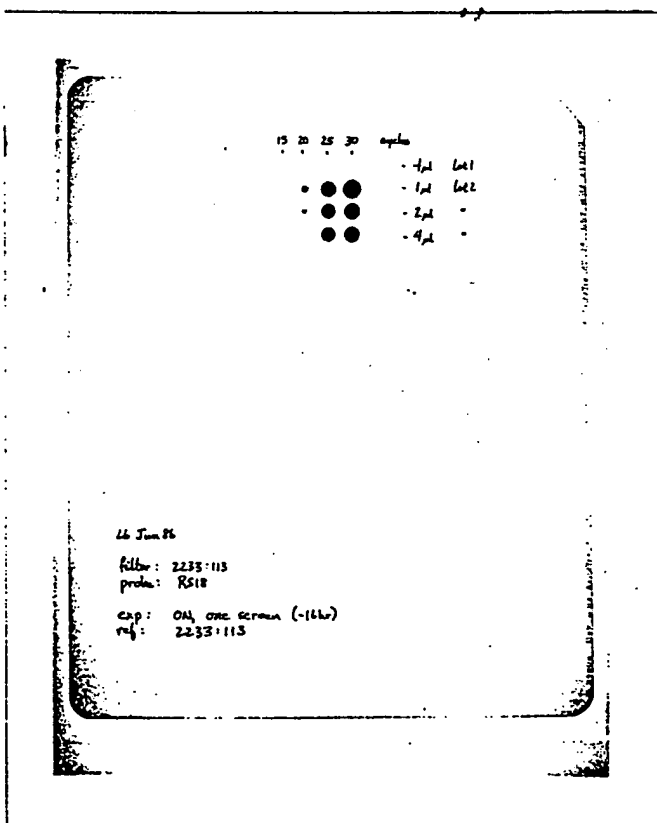
RS18 @ 0.057 pmol/ μ l, 0.5 pmol \rightarrow 8.8 μ l (page 107)

Prehyb: 10ml 5xSSPE, 5xDET, 0.5% SDS \rightarrow 15', 55°

Hyb: above + 0.5 pmol RS18 \rightarrow 60', 55°

Wash: 3x 100ml 2xSSPE, 0.1% SDS \rightarrow 5-10', RT each

1x 100ml 5xSSPE, 0.1% SDS \rightarrow 5', 60°



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R. Saito

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Repeat Tag pol assay essentially as described page III except ~~with~~ with amounts of enzyme $\leq 1\mu\text{l}$.

A B C D:	1 μl	(lot 2)		
E F G H:	1/2 μl	"	A, E, I, M, Q, U:	15 cycles
I J K L:	1/4 μl	"	B, F, J, N, R, V:	20 "
M N O P:	1/8 μl	"	C, G, K, O, S, W:	25 "
Q R S T:	1/16 μl	"	D, H, L, P, T, X:	30 "
U V W X:	1/32 μl	"		

Molt4 @ 100 $\mu\text{g}/\text{ml}$, PC03 and PC04 @ 5 μM , dNTP @ 40 mM , gelatin @

80 μl	DNA	
80 μl	10x salts	Divide into five 100 μl and one 200 μl
80 μl	gelatin	samples and heat $\rightarrow 10', 98^\circ$
80 μl	PC03	Cool to RT and to 200 μl sample
80 μl	PC04	add 2 μl Tag pol (lot 2).
80 μl	DMSO	Prepare five 2-fold 100 μl serial
105 μl	dNTP	dilutions in remaining 5 tubes.
200 μl	H ₂ O	(range: 1 μl to 1/16 μl
800 μl		Tag pol per tube)
		Sub-divide each further into four
		25 μl samples and overlay with
		mineral oil (total: 24 tubes)

Subject to 14-29 cycles :

2 1/2' @ 98°
3' @ 37°

← new program
3' @ 37° instead of 5
old

After last cycle incubate additional 10' @ 60° to finish off final extension.

Extract mineral oil with CHCl₃. Store at -20°

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Hybridize Southern of Tag polymerase assay with RS18.

RS18 @ 0.057 pmol/ μ l, 0.5 pmol \rightarrow 8.8 μ l (page 107)

Prehyb: 20ml 5xSSPE, 5xDET, 0.5% SDS \rightarrow 45', 55°

Hyb: above + 0.5 pmol RS18 \rightarrow 90', 55°

Wash: 3x100ml 2xSSPE, 0.1% SDS \rightarrow ~5', RT each

1x100ml 5xSSPE, 0.1% SDS \rightarrow ~15', ~~55~~ 60°

Hyb: 2233:113, 117 with RS18

TITLE Dot Blot: Tag Pol Assay (Lot2) - II

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Prepare dot blot containing PCR samples from Tag polymerase assay with $\leq 1 \mu\text{l}$ enzyme per reaction.

Samples described page 115.

Apply 5 μl each sample to filter:

5 μl	PCR sample
195 μl	0.4 N NaOH, 25 mM
200 μl	

Work-up as described page 113.

Label filter 2233:117

~~Hybridize RS18 to above filter along with sample~~
~~2233:113~~

Hybridize RS18 to filter above plus 2233:113 as positive control.

RS18 @ $0.057 \text{ pmol}/\mu\text{l}$, $0.5 \text{ pmol} \rightarrow 8.8 \mu\text{l}$ (p107)

Prehyb: 10 ml 5xSSPE, 5xDET, 0.5% SDS \rightarrow 15', 55° (both filter in same bc)

Hyb: above + 0.5 pmol RS18 \rightarrow 3hr, 55°

Wash: 3x 100 ml 2xSSPE, 0.1% SDS \rightarrow ~5', RT each
1x 100 ml 5xSSPE, 0.1% SDS \rightarrow 20', 60°

